gemcitabine, or irinotecan is probably acceptable as the current standard first-line chemotherapy.

First-line single agent with gefitinib is active, but produces unacceptably frequent ILD in the Japanese population. Being female, as well as adenocarcinoma, those who never smoked, and *EGFR* mutation were associated with response to gefitinib. Patients who responded to gefitinib did not experience ILD during gefitinib chemotherapy. Further research via genetics and image analysis is needed to avoid ILD and identify a subgroup of patients that benefit from gefitinib treatment. If this is realized, single agent treatment with gefitinib could be an option as first-line chemotherapy in selected patients with advanced NSCLC. Furthermore, randomized trials are warranted to compare first-line single agent treatment with gefitinib followed by second-line platinum-based chemotherapy with first-line platinum-based chemotherapy followed by second- or third-line gefitinib treatment.

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The authors indicated no potential conflicts of interest.

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Original contribution

Gemcitabine/Carboplatin in a Modified 21-Day Administration Schedule for Advanced-Stage Non–Small-Cell Lung Cancer

Mana Yoshimura, Fumio Imamura, Kiyonobu Ueno, Junji Uchida

Abstract

PURPOSE: Gemcitabine/carboplatin is active for advanced-stage non–small-cell lung cancer. Although it has a better toxicity profile than gemcitabine/cisplatin, severe thrombocytopenia can be a problem. We conducted a phase II study of gemcitabine/carboplatin on a 21-day schedule with administration of carboplatin delayed until day 8, intending to decrease the severity of thrombocytopenia and evaluate the feasibility and efficacy of this schedule. PATIENTS AND METHODS: Thirty-one patients with stage IIIB or stage IV non–small-cell lung cancer received gemcitabine 1000 mg/m^2 on days 1 and 8 and carboplatin at an area under the curve of 5 mg × minute/mL on day 8, every 21 days. RESULTS: The response rate was 22.6%, including 1 complete response. The median time to progression was 161 days, and the median survival was 454 days. Grade 3/4 thrombocytopenia, according to the National Cancer Institute Common Toxicity Criteria, version 3.0, was observed in 2 patients (6.5%) in the first 2 cycles. Nonhematologic toxicity included rash, depression, fever, nausea/vomiting and increased hepatic transaminase. The median courses of delivery were 3, and 13 patients (42%) received the first 3 courses without treatment delay. Dose intensity for each drug was 638 mg/m^2 per week for gemcitabine and 1.56 mg × minute/mL per week for carboplatin area under the curve, respectively. CONCLUSION: This study suggests that gemcitabine/carboplatin with a day-8 administration of carboplatin in a 21-day schedule reduces the severity of thrombocytopenia without having a detrimental effect on efficacy.

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Key words: Dose intensity, Feasibility, Phase II studies, Thrombocytopenia

Introduction

Non–small-cell lung cancer (NSCLC) constitutes 75%-80% of lung cancer cases and currently represents a leading cause of cancer-related death throughout the world.[1] Significant proportions of the patients present with locally advanced or metastatic disease at the time of diagnosis.[2] Although a recent overview suggested that platinum agent–based chemotherapy improves survival and quality of life,[3] the long-term prognosis of these patients is still generally poor. In the past 2 decades, several new chemotherapeutic agents have been developed and have proven to be active in advanced-stage NSCLC. Gemcitabine, a pyrimidine antimetabolite, is one of the most promising among these agents, showing definite efficacy and mild toxicity profiles. Initial phase I studies using a schedule of weekly administrations of 3 weeks for every 4 weeks established 790 mg/m^2 weekly as the maximum tolerated dose. Dose-limiting toxicity was myelosuppression, with thrombocytopenia more significant than granulocytopenia.[4] Later phase I/II studies have established 1250 mg/m^2 weekly as an optimal tolerated dose.[5-7] Several phase II studies of single-agent gemcitabine in advanced-stage NSCLC have demonstrated response rates of 20%-26% and a median survival of 7-9.4 months.[8-13] In these studies, 800-1250 mg/m^2 gemcitabine was administered weekly for 3 weeks every 4 weeks. Toxicities reported in these studies were myelosuppression, such as granulocytopenia and thrombocytopenia, transient increase of hepatic transaminases, rash, flu-like symptoms, and lethargy.

The combination of gemcitabine and a platinum compound has demonstrated a synergistic effect in preclinical settings, and a number of phase II/III studies of gemcitabine/cisplatin have been performed.[14-22] This combination chemotherapy has proved to be very promising, showing

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an objective response rate (ORR) of 28%-54% and a median survival of 8.4-15.4 months. Gemcitabine/cisplatin is now one of the standard chemotherapy combinations for advanced-stage NSCLC. However, the toxicity profile of cisplatin, such as nausea/vomiting, nephrotoxicity, and neurotoxicity, can be troublesome for patients with advanced-stage NSCLC, who generally have poor prognosis. Moreover, cisplatin is often intolerable for certain patients, especially the elderly and/or those with concomitant severe diseases. Carboplatin is a cisplatin analogue, and its nonhematologic toxicity is milder compared with cisplatin. Carboplatin is also expected to exert a synergistic effect with gemcitabine. Several phase II studies of gemcitabine/carboplatin have been reported. The early studies adopted a schedule of weekly administration of gemcitabine for 3 weeks (day 1, 8, and 15 administrations) and day-1 administration of carboplatin every 4 weeks.[23-29] However, those studies reported high incidences of thrombocytopenia, prompting the investigation of other schedules that are less myelosuppressive. Iaffaiolli et al recommended a 28-day schedule that decreased myelotoxicity around day 15 by administrating carboplatin on day 8 and eliminating the administration of gemcitabine on day 15.[30] Edelman et al recommended a 21-day schedule that decreased myelotoxicity around day 15 by simply eliminating the administration of gemcitabine on day 15.[31] Several large phase II studies have been performed using these schedules. Among them, Mott et al reported a phase II study with a 28-day schedule described by Iaffaiolli et al, with an ORR of 10% and a median survival of 8.3 months.[32] On the other hand, Yamamoto et al reported the results of a comparative phase II study in which a 21-day schedule described by Edelman et al was compared with gemcitabine/vinorelbine as a control arm.[33] The ORR of gemcitabine/carboplatin was 20%, and the median survival of 432 days was favorable. However, a high incidence of dose reduction as a result of myelosuppression and early withdrawal from the study were reported. These studies suggest that the schedule for gemcitabine/carboplatin still needs improvement. In the present article, we report another 21-day schedule, with the intent to be more dose intense than Mott et al and less myelosuppresive than Yamamoto et al.

Patients and Methods

Eligibility Criteria

Eligibility criteria of patients were as follows: age 20-80 years, a histologic or cytologic diagnosis of clinical stage IIIB NSCLC with malignant pleural effusion or clinical stage IV NSCLC, and Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0-2. Patients were required to have adequate bone marrow reserve (leukocyte count > 4000/μL, platelet count > 100,000/μL, and hemoglobin > 10 g/dL), normal hepatic function (serum bilirubin < 1.5 mg/dL, transaminases < 2 times the upper limit of normal), normal renal function (serum creatinine < 1.2 mg/dL), and a life expectancy of > 3 months. Patients who did not have measurable disease based on Response Evaluation Criteria in Solid Tumors[34] were excluded from the study. Neither previous chemotherapy nor thoracic irradiation was allowed. Patients were excluded from the study when they met one of the following conditions: active uncontrolled infection, unstable concomitant disease (ischemic heart disease, hypertension, or diabetes mellitus), active concomitant malignant disease, pregnancy, or breastfeeding. Written informed consent was obtained from all patients.

Study Design

This was a single-arm phase II study. Because the response rate of gemcitabine/carboplatin has been reported by a variety of authors, we determined the primary endpoint of our study as the rate of treatment completion without treatment delay. It has been reported that the median courses of delivery of platinum-doublet chemotherapy was approximately three[35] and that there was no statistical significance in survival of patients between 3 and 6 courses of platinum agent–containing chemotherapy.[36] Therefore, we analyzed drug delivery in the first 3 courses to evaluate the feasibility of the schedule and defined the treatment completion rate to be the percentage of patients who received the first 3 courses with no delay from the intended schedule. The expected and threshold value of the treatment completion rates were 90% and 70%, respectively. The number of patients required was determined with an α risk of 0.05 and a β risk of 0.2. Simon's optimal design was applied to recruit the patients[37]: if completion of treatment was observed in < 5 patients among the first 6 patients, the study was to be terminated; if it was observed in ≥ 5 patients, recruitment of as many as 27 patients was allowed. This schedule was judged to be feasible when, in an analysis of 27 patients, treatment completion was observed in > 22 patients. The secondary endpoints included the evaluation of response rate, toxicities, median time to progression (TTP), and overall survival. This study was approved by the Institutional Review Board of Osaka Medical Center for Cancer and Cardiovascular Diseases.

Treatment Plan

Patients received carboplatin at an area under the curve (AUC) of 5 mg × minute/mL, calculated using the Calvert formula[38] with creatinine clearance evaluation by the Cockcroft formula.[39] Carboplatin was administrated in a 60-minute infusion on day 8 of a 21-day cycle. Gemcitabine was administrated at 1000 mg/m^2 in a 30-minute infusion on days 1 and 8. The planned dose intensity for each drug was 667 mg/m^2 per week for gemcitabine and 1.67 mg × minute/mL every week for carboplatin AUC. Four cycles of treatment were intended. On day 1 and day 8 of each cycle, complete blood count was evaluated. Drug administration was delayed until recovery in cases with leukocyte count < 3000/μL or platelet count < 100,000/μL on day 8.

The hematologic criteria to start the next cycles were loosened to increase dose intensity (leukocyte count < 2500/μL,

Table 1 Patient Characteristics (N = 31)

Characteristic	Number of Patients
Median Age, Years (Range)	63 (42-76)
Sex	
Male	12
Female	19
Stage	
IIIB	8
IV	23
Histology	
Adenocarcinoma	25
Squamous cell carcinoma	6
ECOG PS	
0	22
1	9

and platelet count > 75,000/μL). The start of the new cycles was postponed until blood count met these criteria. Doses of gemcitabine were adjusted according to leukocyte, neutrophil, and platelet counts. If grade 4 leukopenia or neutropenia continued > 3 days despite the use of granulocyte colony-stimulating factor or if platelet count decreased to < 25,000/μL, the gemcitabine dose was reduced by 200 mg/m^2 intervals until 600 mg/m^2. Patients were withdrawn from the study in cases of disease progression, development of grade > 3 nonhematologic toxicities, unacceptable treatment delay as a result of hematologic toxicities, or necessity of gemcitabine dose reduction to < 600 mg/m^2. After withdrawal from the study, subsequent treatment was to be decided by the investigator.

Evaluation

Response was evaluated by chest and abdominal computed tomography (CT) scans after the second and fourth cycles of chemotherapy according to Response Evaluation Criteria in Solid Tumors. Brain magnetic resonance imaging, chest CT scan, and abdominal CT scan were performed at any time if assessment for the disease progression was necessary. Confirmation was necessary to determine partial and complete response. During the study, all enrolled patients were evaluated weekly by physical examination, complete blood count, and blood chemistries. Toxic effects were graded according to National Cancer Institute Common Toxicity Criteria, version 3.0.

Statistical Analysis

Time to progression was calculated from the date of enrollment to the date of progression using the Kaplan-Meier method.[40] Overall survival was calculated from the date of enrollment until the date of death or last known contact using the Kaplan-Meier method. Statistical analysis in the study was carried out using the SPSS program.

Table 2 Hematologic Toxicities

Adverse Event	Grade 3	Grade 4	N (%)
Leukopenia	10	0	10 (32.2)
Neutropenia	16	5	21 (67.7)
Anemia	3	0	3 (9.7)

Results

From June 2003 to April 2005, 31 eligible patients were enrolled in the study. There were 12 men and 19 women; 6 patients with squamous cell carcinoma and 25 with adenocarcinoma; 8 patients with clinical stage IIIB and 23 with clinical stage IV; 22 patients with an ECOG PS of 0 and 9 with a PS of 1. Sixteen patients had a smoking history. Patient characteristics are summarized in Table 1. Tumor response was assessable in all 31 patients. One complete response and 6 partial responses were observed, resulting in a response rate of 22.6%. Median TTP was 161 days (95% confidence interval, 109-213 days). At the time of analysis, when the median follow-up time was 356 days (range, 40-946 days), 12 patients were alive, 16 patients were dead, and 3 patients were lost to follow-up. Median survival time was 454 days (95% confidence interval, 230-678 days).

Toxicity profiles are summarized in Tables 2, 3, and 4. Table 2 shows hematologic toxicities except thrombocytopenia in the first 2 cycles. Neutropenia was frequently observed, with grade 3/4 neutropenia occurring in 51.6% (16 of 31 patients) and 16.1% (5 of 31 patients) of the patients, respectively. However, febrile neutropenia was not observed. Grade 3 anemia was observed in 9.7% of patients (3 of 31 patients), and grade 4 anemia was not observed. The incidence of red blood cell and platelet transfusions was 3.2% (1 of 31 patients) and 3.2% (1 of 31 patients), respectively. Because the grading of thrombocytopenia is substantially different among versions of the National Cancer Institute Common Toxicity Criteria, we show detailed results of platelet numbers in Table 3. Thrombocytopenia was relatively mild; grade 3/4 thrombocytopenia occurred in 3.2% (1 of 31 patients) and 3.2% (1 of 31 patients) of patients in the first 2 cycles, without serious hemorrhagic events. The lowest platelet count was 15,000/μL and was observed in the first cycle in a 74-year-old man. Grade 2/3 nausea/vomiting occurred in 3.2% (1 of 31 patients) and 3.2% (1 of 31 patients) of patients, respectively, grade 2 and 3 rash in 6.5% (2 of 31 patients) and 12.9% (4 of 31 patients), grade 3 depression in 3.2% (1 of 31 patients), grade 1 fever (in the absence of neutropenia) in 3.2% (1 of 31 of patients), and grade 1 hepatic transaminase increase in 9.7% (3 of 31 patients). A total of 94 cycles with a median of 3 cycles for each patient were administered. Treatment was delayed in 42.6% of cycles and required dose reduction in 6.4% of cycles. The median number of days per cycle was 24 days (22, 29, and 26 days for the first, second, and third cycles, respectively). The dose intensity was 638 mg/m^2 per week for gemcitabine and 1.56 mg × minute/mL per week for carboplatin AUC.

Table 3 Thrombocytopenia Incidence

Thrombocytopenia	N	Overall	1/2 Cycles	≥ 3 Cycles
Grade 3/4	31	2/3 (16.2%)	1/1 (6.5%)	1/2 (9.7%)

Nadir platelet counts in 5 cases with grade > 3 thrombocytopenia ($\times 10^4$) were 1.5, 2, 2.5, 3.9, and 4.9.

Table 4 Nonhematologic Toxicities

Adverse Event	Grade 1	Grade 2	Grade 3	Grade 4
Nausea	2	1	1	0
Rash	0	2	4	0
Depression	0	0	1	0
Fever (Absence of Neutropenia)	1	0	0	0
Transaminase	3	0	0	0

Among the first 6 patients, 5 had ≥ 3 treatment cycles without treatment delay (4, 3, 2, 8, 4, and 4 cycles for the first, second, third, fourth, fifth, and sixth patients, respectively). Final analysis revealed that 21 of 31 patients received ≥ 3 treatment cycles, but 8 of these patients experienced treatment delay in the first 3 cycles. The treatment completion rate was not sufficiently high at 42%. Ten patients were withdrawn from the study early; the reason for withdrawal was progressive disease for 2 patients, hematologic toxicity for 3 (all were neutropenic but did not have thrombocytopenia), and nonhematologic toxicity for 5 (grade 3 depression in 1 patient and grade 3 rash in 4 patients; 1 was caused by carboplatin, and the others were caused by gemcitabine).

Discussion

Third-generation chemotherapy, consisting of a platinum agent and a third-generation chemotherapeutic agent, including gemcitabine, is considered a standard treatment for advanced-stage NSCLC worldwide. Many studies were carried out to compare the toxicity and efficacy of each regimen of third-generation chemotherapy. According to the ECOG 1594 study, a significant difference in efficacy is difficult to demonstrate among the regimens.[41] In contrast, the profiles of toxicities were demonstrably different among the regimens.

Although platinum compounds, such as cisplatin and carboplatin, are still key drugs in chemotherapy for NSCLC, a recent metaanalysis suggested that treatment with regimens containing gemcitabine showed small but statistically significant improvement in patient survival.[42] With its mild toxicity and easiness in administration, gemcitabine is becoming another key drug in chemotherapy for NSCLC. In a Japanese phase III trial in which gemcitabine/vinorelbine/paclitaxel in combination with a platinum agent were compared with irinotecan/cisplatin, a Japanese standard for NSCLC, gemcitabine/cisplatin exerted the best result; however, the difference was not statistically significant.[35] Recent trials showed that the gemcitabine/carboplatin improved patient survival compared with gemcitabine alone and mitomycin/ifosfamide/cisplatin.[43,44] Taking these results together, gemcitabine/carboplatin is a reasonable combination and becoming widely used for NSCLC.

Early studies of gemcitabine/carboplatin used a 28-day schedule in which gemcitabine was administered on days 1, 8, and 15 and carboplatin was administered on day 1.[23-29] However, because of a high incidence of severe thrombocytopenia, 2 alternate schedules were proposed: one is a 21-day schedule treatment in which gemcitabine is administered on days 1 and 8 with carboplatin administered on day 1,[31] and the other is a 28-day schedule in which gemcitabine is administered on day 1 and 8 with carboplatin on day 8.[30] Obasaju et al conducted a randomized phase II study comparing these 2 schedules.[45] Although the study was not powered to show a statistically significant difference between these 2 regimens, the 21-day schedule seemed to be superior to the 28-day schedule in terms of efficacy. However, grade 3/4 thrombocytopenia was observed in 14% of cycles in the 21-day schedule, higher than that in the 28-day schedule. The 21-day schedule has been used in several other studies, in which thrombocytopenia was still the main problem, accompanied by bleeding episodes, although not frequently.[27,46,47] In the Japanese phase II study described previously, thrombocytopenia was again a major issue, resulting in a high incidence of dose reduction and early withdrawal from the study.[33] Nevertheless, good median survival time of the patients treated with gemcitabine/carboplatin (432 days) and low incidences of nonhematologic toxicities were impressive. Meanwhile, the 28-day schedule in which carboplatin was administered on day 8 appeared to be less myelotoxic than the 21-day schedule but has the problem of low dose intensity.

Our study was designed to evaluate the feasibility and efficacy of gemcitabine/carboplatin in a modified administration schedule. Gemcitabine/carboplatin were administered at 1000 mg/m^2 on days 1 and 8 and at AUC 5 on day 8 of each 21-day cycle, respectively. The main aim of this study was to decrease the severity of thrombocytopenia with minimal effect on dose intensity. The low incidence of grade 3/4 thrombocytopenia was notable, observed in only 2 of 31 patients in the first 2 cycles. This result suggested that the nadir of thrombocytopenia of gemcitabine and carboplatin occur around day 15, and that incidence of severe thrombocytopenia could be decreased even in a 21-day schedule by delaying administration of carboplatin until day 8. We were concerned whether this 3-weekly chemotherapy would become possible by adopting looser criteria (leukocyte count > 2500/μL and platelet count > 75,000/μL) to start new cycles. Other hematologic and nonhematologic toxicities were also mild, and altogether, the treatment was well tolerated. The incidence of stressful toxicities represented by nausea/vomiting, neurologic toxicities, and alopecia was relatively low in the gemcitabine/carboplatin combination.

The planned dose intensities and actual dose intensities were 667 mg/m^2 per week and 638 mg/m^2 per week (95.7% of planned dose intensity) for gemcitabine and 1.67 mg ×

minute/mL per week and 1.56 mg × minute/mL per week (93.4% of planned dose intensity) for carboplatin AUC, respectively. Dose intensity for each drug in the 28-day schedule described previously[30,32] was estimated to be 550 mg/m^2 per week for gemcitabine and 1.25 mg × minute/mL per week for carboplatin AUC, respectively. The median cycles of delivery were 3, which was comparable with those of platinum-doublet chemotherapy.[35] Therefore, our main purpose to decrease the incidence of thrombocytopenia and increase dose intensity was achieved, although there are still problems to be solved.

Drug administrations were frequently delayed, treatment time tended to be protracted, and the treatment completion rate we defined was 42%. Unfortunately, early withdrawal from the study was seen in 10 patients (32%). Among these patients, 3 experienced grade > 2 leukopenia (leukocyte count < 3000/μL) on day 8 of the first course, and the other 3 patients developed grade 3 rash after administration of day 1 gemcitabine. For these 6 patients, gemcitabine/carboplatin chemotherapy was considered inappropriate regardless of the schedule. This schedule, which delays carboplatin administration until day 8, would enable early exclusion of the patients who are inappropriate for this combination chemotherapy, avoiding severe hematologic and nonhematologic toxicities. Response rate, median TTP, and median survival time were favorable. However, this might be biased by the small number of patients and the high percentage of patients with good prognostic factors such as female sex and PS of 0 in this study.

Recently, prolonged administration of gemcitabine combined with carboplatin has been tested.[48,49] Because gemcitabine/carboplatin combination chemotherapy has become a widely used regimen, further improvement of this regimen is necessary.

Conclusion

The present study suggests that carboplatin administered on day 8 in a 21-day schedule of gemcitabine/carboplatin reduces severity of thrombocytopenia without having a detrimental effect on efficacy. However, further evaluation is still needed to estimate the efficacy and feasibility of this regimen. The ongoing randomized phase II study compares day-1 and day-8 administration of carboplatin in a 21-day schedule of gemcitabine/carboplatin. In clinical practice, this regimen will be one of the treatment options suitable for outpatients.

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Genome-wide cDNA microarray screening of genes related to the benefits of paclitaxel and irinotecan chemotherapy in patients with advanced non-small cell lung cancer

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Previous studies have demonstrated that not only the benefits but also the toxicities of chemotherapy can be predicted by cDNA microarray analysis of tumor specimens obtained before chemotherapy against non-small cell lung cancer (NSCLC). We conducted a study of cDNA microarray analysis to determine whether the gene expression in peripheral blood taken from patients prior to chemotherapy were correlated with the outcome of chemotherapy with paclitaxel (Pac) and irinotecan (CPT) against advanced NSCLC . Thirty-one patients with stage IIIB or IV NSCLC were treated with CPT at 60 mg/m^2 and Pac at 160 mg/m^2 every 2 weeks. Seventeen of 31 patients achieved PR and the overall RR was 54.8%. The median survival time was 426 days and the 1-year survival rate was 58.1%. The expression levels of 1176 genes were analyzed in 31 patients with the AtlasTM Human Cancer 1.2 Array. Stepwise multivariate analysis revealed that the genes encoding protein phosphatase, IL-1α and IgA were independent predictive factors for chemosensitivity. Stepwise regression analysis revealed that the thyrotropin-releasing hormone receptor and alkylation repair genes were independent prognostic factors. In conclusion, the expression of certain genes was able to predict the benefits of this Pac and CPT chemotherapy regimen.

Key words: microarray, paclitaxel, irinotecan, lung-cancer, gene

INTRODUCTION

Current chemotherapy regimens for metastatic non-small cell lung cancer (NSCLC) are not particularly effective, and the disease cannot be cured even with the most effective chemotherapy. Responders to chemotherapy may have a better prognosis than non-responders (1) and chemosensitivity is an important factor in deciding which patients should receive chemotherapy in such non-curative NSCLC. Previous study has demonstrated that not only the benefits but also the toxicities of chemotherapy can be predicted by cDNA microarray analysis of tumor specimens obtained before chemotherapy (2). The results suggest that the intrinsic genetic characteristics of individual patients will reflect the outcomes of chemotherapy and lead to the hypothesis that genetic analysis of non-malignant cells can also be used to predict the benefits and toxicities of chemotherapy.

Our previous phase I study of a paclitaxel (Pac) and irinotecan (CPT) combination led to a recommendation of Pac 160 mg/m^2 and CPT 60 mg/m^2 every 2 weeks for further study (3). This study also demonstrated an objective response rate of 58.3%, and a 1-year survival rate of 54.2%. Accordingly, we examined the correlations between gene expression in peripheral blood, which is easily available, and the benefits of the combination chemotherapy with Pac and CPT to display high activity against NSCLC.

Table 1. *Patient characteristics*

No. of patients		
Total		31
Age, years	Median	61
	Range	43 – 69
Gender	Male	20
	Female	11
Performance status	0	9
(ECOG)	1	22
Clinical stage	IIIB	5
	IV	26
Histology	Adenocarcinoma	24
	Others	7

PATIENTS AND METHODS

The Institutional Review Board of Kanagawa Cancer Center reviewed and approved this study prior to commencement.

Patients. Patients with histologically or cytologically confirmed NSCLC were registered. Eligibility criteria were: clinical stage IIIB or IV, age <70 years, Eastern Cooperative Oncology Group PS score ≤1. Patients who had received chemotherapy or radiotherapy were excluded from this study. Written informed consent was obtained from every patient.

Chemotherapy. All patients without disease progression were treated every 2 weeks for a total of four courses of chemotherapy. CPT was administered at a dose of 60 mg/m^2 on day 1. Pac was administered at a dose of 160 mg/m^2 on day 1. Premedication consisting of 20 mg dexamethasone and 50 mg ranitidine was infused. A 50 mg oral dose of diphenhydramine was also administered. Prophylactic G-CSF, 50 μg/m^2/day or 2 μg/kg/day, was administered subcutaneously on days 6 to 10. Patients were given a 5-HT$_3$ antagonist intravenously. Tumor response was evaluated according to RECIST criteria (4).

Blood samples, purification of RNA and cDNA microarray. Genomic DNA was obtained from peripheral blood mononuclear cells (PMNC) isolated from 10 ml of peripheral blood taken from patients prior to chemotherapy. The total RNA of each sample was isolated and treated with DNase I to avoid contamination by genomic DNA by using silica membrane affinity chromatography and a total RNA isolation kit (Macherey-Nagel GmbH & Co., KG, Germany). One hundred nanograms of the total RNA for each sample was reverse transcribed into cDNA. Each cDNA sample was subjected to microarray expression profiling with the BD Atlas™ Human Cancer 1.2 Array (Clontech) (2). Each labeled probe was then hybridized into a separate Atlas Array. The signal intensity for each spot, which corresponds to each gene examined, was determined with a STORM image analyzer (Amersham Bioscience, Picataway, NJ). The hybridization pattern and signal intensity were analyzed to determine changes in gene expression levels by using AtlasImage™ 2.01 software (Clontech Laboratory Inc., Japan).

Statistical methods. The association between gene expression and tumor regression during chemotherapy was tested with the Pearson correlation coefficient. To determine whether gene expression profiles were associated with differences in survival, Kaplan-Meier survival plots and log-rank tests were used. The influence of expression of each gene on chemotherapy outcomes was examined by stepwise multivariate regression analysis or cox proportional hazards model analysis. $P < 0.05$ was considered significant.

RESULTS

Between May 2002 and July 2004, 31 patients were registered in the study (Table 1). Twenty-seven patients received 4 to 6 cycles of chemotherapy, except for 4 patients who discontinued treatment in the first or second cycles because of disease progression in 3 patients and grade 2 pneumonitis in 1 patient. Seventeen of 31 patients achieved PR, 10 NC and 4 PD, and the overall RR was 54.8% in this study. The median survival time was 426 days and the 1-year survival rate was 58.1%.

The expression levels of 1176 genes in the peripheral blood cells of 31 patients were analyzed by cDNA microarray screening. Four housekeeping genes that were expressed in all 31 samples were used as controls for gene expression: ubiquitin, liver glyceraldehyde 3-phosphate dehydrogenase, 23-kDa highly basic protein, 60S ribosomal protein L13A and 40S ribosomal protein S9.

Stepwise multivariate analysis revealed that protein phosphatase with EF-hands-2 long form, IL-1α and IgA 1 heavy chain constant region + IgA2 heavy chain constant region were independent predictive factors for chemosensitivity ($p < 0.001$, Table 2). Of these genes, expression of protein phosphatase and IL-1α was positively, and expression of IgA was negatively, correlated with tumor regression rate. When we analyzed the relationship between gene expression levels and survival, the expressions of 10 genes were significantly correlated with survival times ($p < 0.01$). Stepwise regression analysis revealed that thyrotropin-releasing hormone receptor and alkylation repair genes were independent prognostic factors ($p < 0.01$, Table

Table 2. Genes closely associated with sensitivity or survival in chmeotherapy.

	Description	coefficient	P
Sensitivity	protein phosphatase with EF-hands-2 long form	-0.436	0.0134
	IL-1 alpha	-0.432	0.0145
	IgA 1 heavy chain constant region+ IgA 2 heavy chain constant region	0.463	0.008
Survival	thyrotropin-releasing hormone receptor	0.509	0.0029
	alkylation repair; alkB homologue	0.489	0.0046

Stepwise multivariate analysis for sensitivity and stepwise regression analysis for survival were used.

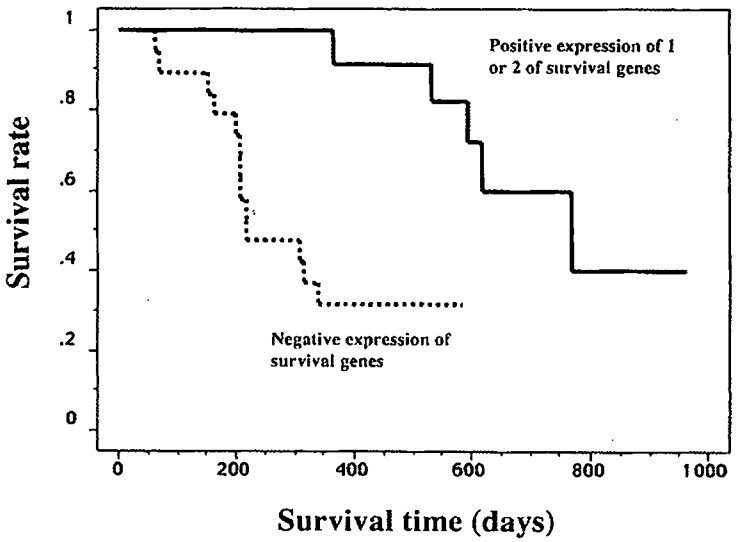

Figure 1. Survival curves constructed by the Kaplan-Meier method. The 12 of the 31 patients who showed positive expression of either the thyrotropin-releasing hormone receptor or alkylation repair genes had a significantly better chance of survival (log-rank, p = 0.0024; Wilcoxon, p = 0.0016)

2). The 12 of the 31 patients who showed positive expression of either thyrotropin-releasing hormone receptor or alkylation repair genes had a significantly better chance of survival (log-rank, p = 0.0024; Wilcoxon, p = 0.0016; Fig. 1). Cox proportional hazards model demonstrated that positive expression of these genes was only significantly dependent prognostic factor (p=0.0094, Table 3).

DISCUSSION

We previously reported that examination of tumor tissues revealed a number of genetic predictors not only of beneficial but also of toxic effects of cancer chemotherapy (2). The fact that genetic information from tumor cells can predict not only tumor susceptibility to chemotherapy but also toxicity suggests that certain genetic characteristics may be common to all somatic cells, irrespective of whether they are malignant or normal. To add support for this hypothesis, in this study we used peripheral blood cells as non-malignant normal cells for analysis of informative genetic factors that can predict the antitumor effects. Protein phosphatase, IL-1α and IgA were predictors of sensitivity to Pac and CPT combination chemotherapy. The adenoviral type 5 E1A protein has been shown to induce sensitization to apoptosis induced by different categories of anticancer drug. Up-regulation by E1A of the catalytic subunit of protein phosphatase 2A in human breast cancer cells was shown to enhance the activity of the phosphatase, which resulted in repression of Akt

Table 3. Cox Proportional Hazards Model for Survival Analysis in paclitaxel and irinitecan treatment.

		Hazard Ratio	95% CI	P
Gender	Female/Male	0.701	0.127-3.86	0.6833
Performance status	0/1	0.706	0.173-2.872	0.6264
Stage	IIIB/IV	0.247	0.030-2.024	0.1926
Hb		0.956	0.534-1.714	0.8803
Albumin		0.405	0.109-1.504	0.1770
LDH		1.002	0.997-1.006	0.4442
Survival gene	Negative/Positive	9.102	1.720-48.180	0.0094

activation in E1A-expressing cells (5). This up-regulation of protein phosphatase 2A might represent a novel mechanism for E1A-mediated sensitization to anticancer drug-induced apoptosis. IL-1α is a cytokine with many activities central to immune function and hematopoesis. This cytokine dramatically increases the sensitivity of osteosarcoma cells to etoposide when the two agents are used simultaneously (6). Thyrotropin-releasing hormone (TRH) receptor and alkylation repair genes were identified as independent prognostic factors. TRH plays a key role in the regulation of the thyroid axis. A number of changes in hormonal secretion patterns have been found in subjects with neoplastic disease. When mean nocturnal levels were compared, cortisol, TRH and growth factor levels were higher in patients with lung cancer than in normal controls (7). TRH and its receptor are also expressed in non-hypothalamic cells such as pancreatic cells, suggesting that TRH might play a biological role in an autocrine fashion (8). It is possible that a TRH-related autocrine system in normal cells may overcome the cachexia induced by lung cancer.

The development of cancer involves the concurrent disruption of regulation of expression of multiple genes. Therefore, DNA repair systems play an important role in tumor growth and patient survival. The acquisition of methylation of the DNA mismatch repair gene hMLH1 in plasma DNA after chemotherapy predicts poor survival for ovarian cancer patients (9), suggesting that depression of the repair system increases tumor growth and decreases patient survival time. It therefore appears reasonable that the present study showed that increased expression of alkylation repair genes is correlated with good survival.

We need to undertake prospective evaluations to determine whether the genes revealed in this study are truly important and potentially useful for predicting the beneficial of chemotherapy. Accumulation of such data could eventually allow chemotherapy to become "personalized", allowing the use of anticancer drugs that are effective in individual patients.

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ELSEVIER

Seminars in **Oncology**

Geographic Variation in the Second-Line Treatment of Non–Small Cell Lung Cancer

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Although there is broad agreement on management options for treating different stages of non–small cell lung cancer (ie, surgery for stage I and II disease, combined treatment modalities for stage III disease, and platinum-based chemotherapy as initial treatment for appropriate patients with stage IV disease), there is considerable geographic variation in practice patterns. These variations reflect a number of factors, including health care economics, the influence of national and regional regulatory bodies, the nature of physician and patient interaction, and probable biological differences between different populations in terms of drug metabolism and inherent susceptibility to both drug activity and toxicity. The approaches taken by three different geographic regions, the United States, European Union, and Japan, are evaluated. Clinically, the most striking differences in activity and toxicity between different regions have been seen with the epidermal growth factor receptor inhibitors gefitinib and erlotinib. Japanese patients experience significantly greater response and a greater degree of interstitial lung disease than patients in the European Union and North America (ie, US and Canada). Similar differences in efficacy and toxicity have also been noted with cytotoxic chemotherapy agents in the first-line setting. These geographic and ethnic differences in toxicity and efficacy will need to be considered in the design and comparison of future clinical trials.
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Lung cancer is the most lethal malignancy in the developed world, and was expected to account for over one million deaths worldwide in 2005.[1] Non–small cell lung cancer (NSCLC) accounts for approximately 85% of these cases.[2] The vast majority of cases are secondary to tobacco use. Other etiologies include asbestos and radon exposure as well as a genetic contribution.

Although standards of care have been established for different stages of the disease, there is considerable geographic variation in practice patterns. Three major geographic factors influence the choice of second- and third-line therapy. First is the influence of the regulatory agencies that govern the approval of antineoplastic agents. Second is the influence of the specific national healthcare system, including factors governing reimbursement to patients and physicians for treatment. Finally, and most significantly, is the emerging recognition that there are biological differences between different populations in terms of drug metabolism and inherent efficacy. This article will briefly review the approaches taken to second-line therapy in three different areas of the world: the United States, European Union (EU), and Japan.

Overview of Second-Line Therapy

Docetaxel

The first agent to show unequivocal activity in the second-line treatment of NSCLC was docetaxel. A National Cancer Institute of Canada trial compared docetaxel at 75 mg/m^2 or 100 mg/m^2 versus best supportive care. This trial found superior quality and length of life for patients treated with 75 mg/m^2 docetaxel.[3] An industry-sponsored study in the United States compared docetaxel at either 75 or 100 mg/m^2 versus a physician choice of either vinorelbine or ifosfamide. Again, quality of life and survival were superior for docetaxel 75 mg/m^2.[4] The concordant results of these two trials support

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the conclusion that docetaxel 75 mg/m^2 every 3 weeks has a clear role in this setting. Docetaxel has been approved for treatment of previously treated NSCLC in the United States, EU, and Japan.

Pemetrexed

Pemetrexed, a new antifolate agent that has shown activity in mesothelioma, has been tested in the second-line treatment of NSCLC. A phase III trial randomizing patients to either pemetrexed (500 mg/m^2 every 3 weeks with vitamin B_{12} and folate supplementation) or docetaxel (75 mg/m^2 every 3 weeks) showed a similar level of activity but superior tolerability.[5] There was considerably less myelotoxicity and alopecia in the pemetrexed arm, and significantly fewer patients required hospitalization after treatment than with docetaxel. Activity, in terms of response rate, median survival time, and 1-year survival rate, was superimposable for pemetrexed and docetaxel. Pemetrexed has been approved in the United States and EU for the second-line treatment of advanced NSCLC.

Gefitinib

Gefitinib was the first drug to receive approval for third-line therapy of NSCLC anywhere in the world (Japan). This approval was controversial as its basis was response rate rather than a more unequivocal outcome of patient benefit, such as survival rate.[6] The drug had previously failed to show benefit (in terms of response or survival) as a first-line treatment when combined with standard chemotherapy.[7,8]

Two large phase II trials of gefitinib monotherapy, the Iressa Dose Evaluation in Advanced Lung Cancer (IDEAL) 1 and IDEAL 2 studies, evaluated the agent in pretreated NSCLC. Both studies determined response and survival. The IDEAL 1 trial, conducted primarily in Japan and Europe, also evaluated the safety profile and symptom improvement, while the IDEAL 2 trial, conducted in North America, evaluated symptom improvement as an additional primary endpoint.[9,10] The response rates for dosages of 250 mg/day and 500 mg/day were 18.4% and 19% in IDEAL 1, and 12% and 9% in IDEAL 2, respectively. Many patients, even those with a poor performance status (ie, performance status 2–3) experienced symptom improvement (most notably in pulmonary symptoms of dyspnea and chest pain) within 2 weeks of starting gefitinib treatment. This improvement in quality-of-life scales, though questionable as there was no randomization against either best supportive care or another agent, was the major impetus for granting conditional approval to market the agent in the United States. Approval was granted under the provision that appropriate randomized trials be conducted. Gefitinib has not received approval in the EU, although it has been approved in Switzerland.

Subset analysis shows that female sex, adenocarcinoma (and, in particular, bronchioloalveolar histology), and nonsmoking status are predictors of response.[10,11] Female sex was a particularly strong predictor in both IDEAL trials. In the primarily North American IDEAL 2 study, 50% of women experienced symptomatic response versus 31% of men ($P = .006$). Radiographic regression was also seen in 19% of women versus only 3% of men ($P = .001$). Two groups in Boston, MA have recently reported that mutations in the aATP-binding pocket of the epidermal growth factor receptor (EGFR) tyrosine kinase (TK) domain predict for clinical benefit from gefitinib.[12,13] While others have confirmed the presence of mutations, the role of mutations versus other alterations in *EGFR* (copy number, expression as measured by fluorescence in situ hybridization) have also been proposed as predictors of response to EGFR TK inhibitors (TKIs). It remains unclear as to whether any of these molecular variables predict independently for outcome.[14]

The role of gefitinib has recently been questioned because of the results of the Iressa Survival Evaluation in Lung Cancer (ISEL) trial.[15] This trial, undertaken in countries in which gefitinib had not received approval (ie, countries other than the United States and Japan) randomized patients between gefitinib and placebo. The ISEL trial was conducted in cooperation with 210 institutes in 28 countries (not including Japan). An advantage was shown in terms of response rate.[15] However, a trend toward improved survival did not achieve statistical significance. The subset analysis in Asian and non-Asian patients showed that female sex and adenocarcinoma histology were more common characteristics in Asian patients (Table 1). The US Food and Drug Administration has recently restricted use of gefitinib to patients who are currently being treated with the agent and who demonstrate benefit, and those enrolled in clinical trials.

Erlotinib

Erlotinib is an agent very similar to gefitinib in terms of structure and activity. It too has been evaluated as a second-line drug in the treatment of NSCLC, showing 'promising results' in terms of response and survival in phase II trials.[16]

However, unlike gefitinib, a phase III trial was unequivocally positive. The National Cancer Institute of Canada led a study (JBR-21) comparing erlotinib with best supportive care in third-line therapy. This large study (more than 700 patients) provided definitive evidence of benefit in terms of survival for this agent.[17] Improvements in response (9% v >1%), median survival (6.7 v 4.7 months; $P < .001$), 1-year survival (31% v 21%), and symptomatology (cough, dyspnea, pain) were observed.[17] Erlotinib has been approved in the United States and EU for the second- and third-line therapy of advanced NSCLC.

Geographic Variations in Treatment

Variations in the efficacy and safety of second-line NSCLC therapies have been observed across geographic regions, and have had an impact on the choice of treatment options within the three key pharmaceutical markets of the United States, the EU, and Japan.

United States

As described above, three agents have been approved by the US Food and Drug Administration for use in the second-line

Table 1 A Comparison of Gefitinib Monotherapy Data Across Geographic Regions

Characteristics	Japanese[40]	Non-Japanese[40]	American[10]	Asian[15]	Non-Asian[15]
No. of patients by gefitinib dose					
250 mg/m²	51	53	102	235	894
500 mg/m²	51	55	114	0	0
Demographics					
Median age (yrs)	60	61	61	61	62
Age range (yrs)	28–77	38–85	30–84	NA	NA
Female (%)	37	22	43	40	31
PS 0–1 (%)	91	83	80	72	64
Stage IV (%)	80	81	89	NA	NA
Adenocarcinoma (%)	76	56	66	64	44
No. of prior chemotherapy regimens (%)					
1	53	59	1	54	48
2	47	41	41	46	52
3 or more	0	0	58	0	0
Treatment efficacy					
Response rate (%)	28	10	10	12	7
Median survival (mos)	12	9.9	6–7	9.5	5.2
1-year survival (%)	50	NA	24–27	44	21
Grade 3–4 toxicity (%)					
Diarrhea	4	3	3	NA	NA
Skin rash	3	5	2	NA	NA
ALT elevation	7	1	1	NA	NA
Interstitial lung disease	2	0	0	2	0.001

Abbreviations: ALT, alanine aminotransferase; NA, not applicable; PS, performance status.

setting: docetaxel, pemetrexed, and erlotinib. Erlotinib also has approval in the third-line setting. Gefitinib, which had been granted an accelerated approval based on the phase II data from the IDEAL studies,[18] has been re-labeled in light of data from the ISEL trial.[19] At present it may only be prescribed in a non-investigational setting for patients who are already receiving the agent and who have demonstrated benefit.

Agent Selection. Controversy exists over which of the three approved agents should be used in the second-line setting. Several factors enter into consideration in the United States. First, docetaxel has also received approval as a first-line agent and is frequently used in this setting with carboplatin or cisplatin. Therefore, a patient who has already received this agent and has progressed would not be a suitable candidate to receive the drug again in the second-line setting. Second, there are no trials comparing the value (in terms of patient benefit) of any of the second-line agents in this setting. As a result, clinical judgement and economic issues are relevant. Third, there appears to be an emerging trend for physicians to use erlotinib in patients who have demonstrated the greatest degree of benefit, ie, non-smokers, women, those patients with adenocarcinoma histology, and those with Asian ancestry. It is possible that selection of patients in the future will also be driven by objective biological markers, ie, the presence of *EGFR* gene mutations or increased *EGFR* copy number. Pemetrexed is therefore used in the remaining population. For most practitioners the superimposable results in terms of survival for pemetrexed and docetaxel, coupled with its superior toxicity profile, make pemetrexed the preferred agent when both drugs are considered for second-line therapy.

Economics. Economic issues are of considerable importance given the expense of the agents. Most insurance programs in the United States will cover the cost of administration of intravenous agents but vary considerably regarding the coverage for oral agents. The cost of gefitinib (USD $2,000 to $3,000/month) is considerable. An assistance program sponsored by the manufacturer is available.

European Union

It is difficult to separate any side effects or outcome differences between the EU countries and North America. Several of the trials described above, including JBR-21 and the randomized trial of pemetrexed versus docetaxel, were conducted with significant accrual from European countries. Approvals within Europe are granted by the European Medicines Agency; a separate Committee for Proprietary Medicinal Products provides clinical expertise for the review process. Pemetrexed, erlotinib, and docetaxel are the agents currently approved in the EU for use as second-line therapy.

Japan

Japan was the first country to approve gefitinib for use in the treatment of lung cancer. Drug approvals in Japan are granted by the Ministry of Health, Labor, and Welfare. The Japanese have a significant preference for oral medications, a factor that is likely to have contributed to the rapid approval of gefitinib.[20]

Approximately 50% of the patients enrolled into the IDEAL 1 trial were Japanese.[9] The remainder were from Europe, Australia, and South Africa, and were predominantly white. Significant differences emerged regarding both efficacy and toxicity; there was no comparison of survival. The response rate was clearly higher for the Japanese (27.5% v 10.4%; $P = .0023$). There were no pharmacokinetic differences to explain this response difference. However, in a multivariate analysis, ethnicity did not emerge as an independent factor for response. Baseline factors such as performance status, sex, and histology appear to explain the ethnic differences.

In the ISEL study, the response rate and median survival time were 12% and 9.5 months in Asian patients and 7% and 5.2 months in non-Asian patients, respectively (Table 1).[15] Mutations of the *EGFR* gene, recently identified in patients with gefitinib-responsive lung cancer,[12,13] correlated well with clinical response to gefitinib and patient survival in retrospective case series studies.[21,22] The relatively high frequency of the mutations in East Asian patients (27% to 34%), compared with 14% or less in American patients, may explain the geographical difference in the efficacy of gefitinib.[12,23] The frequencies of grade 3–4 common toxicities of gefitinib, including diarrhea, skin rash, and alanine transferase elevation, were the same among the study populations (Table 1).

Treatment-Associated Interstitial Lung Disease. Because of the limited number of patients evaluated in clinical trials, it is sometimes difficult to identify and analyze uncommon toxicity before marketing a drug. Interstitial lung disease (ILD) associated with administration of gefitinib came to light in October 2002, 4 months after approval of this agent in Japan.[24] In the IDEAL studies, two Japanese patients developed grade 3–4 ILD (2%), while no patients outside Japan experienced ILD. In the ISEL study, the incidence of grade 3–4 ILD was 2% in Asian patients and .001% in non-Asian patients. In a retrospective evaluation of 112 Japanese patients, the incidence of ILD was 5.4%. The primary risk factor was a prior history of pulmonary fibrosis.[24] Between July 2002 and December 2004, there were 86,800 patients with NSCLC who were estimated to have received gefitinib in Japan. According to the Ministry of Health, Labor, and Welfare 1,473 patients were suspected of having ILD associated with the use of gefitinib and 588 patients died of ILD.[25] A prospective survey of gefitinib toxicity in 3,354 NSCLC patients treated at 698 hospitals in Japan between June and December 2003 showed that the incidence of ILD was 5.8% and the mortality rate was 2.5%.[26] Risk factors for the development of ILD identified in the Japanese population were preceding pulmonary fibrosis, smoking history, poor performance status, and male sex.[24,26,27] ILD tends to appear rapidly after initiation of therapy.[28]

In an analysis by the US Food and Drug Administration comparing the incidence of ILD associated with gefitinib treatment in North America and Japan, there was an incidence of approximately 2% from a Japanese postmarketing experience and 0.3% in approximately 23,000 patients in the United States expanded-access program.[18]

It is interesting to note that ILD has been associated with weekly docetaxel therapy in Japanese patients. In a phase II study, docetaxel as a single agent was administered at a dose of 35 mg/m^2 on days 1, 8, and 15 every 4 weeks in 48 patients with advanced or recurrent NSCLC. Of these, 33 patients had had no prior chemotherapy and 15 had received one prior chemotherapy treatment. Patients who had previously undergone thoracic radiotherapy, who had preceding ILD or pulmonary fibrosis, or who had severe pulmonary emphysema were excluded from the study. Of the 48 patients in the study, five (10.4%) developed grade 3–4 ILD.[29] The incidence of ILD associated with weekly administration of docetaxel in other countries varies with reports: grade 3–4 pulmonary toxicity was noted in seven of 35 (20%) patients in a Spanish study,[30] one of 63 (1.6%) in a French study,[31] none of 110 patients in an Italian study, and none of 30 patients in an American study.[32,33] It is unclear from these data whether the development of ILD represents a toxicity to which Japanese patients are predisposed, or is a diagnosis that is made more frequently in Japan for other reasons.

Differences in Efficacy and Toxicity. The differences between Western populations and the Japanese (and other non-Western ethnicities) in both the efficacy and toxicity of an anticancer agent are an emerging issue. Two recent trials comparing carboplatin plus paclitaxel with other combinations for first-line therapy of NSCLC were conducted in the United States (by the Southwest Oncology Group) and Japan (Japan Cooperative Oncology Group, Four Arm Comparative Study).[34] The carboplatin plus paclitaxel arm was similar in both studies (differing only by a slightly lower dose of paclitaxel in the Japanese study), and criteria for entry, dose modifications, toxicity, and response assessment were identical. Considerable differences in toxicity and activity were noted between the two studies. The rate of febrile neutropenia was five-fold greater (16% v 3%; $P < .0001$) in the Japanese trial, while the rate of neuropathy was substantially lower (5% v 16%; $P = .001$). The response rates were similar, while the 1-year survival rate was better in the Japanese trial (51% v 37%; $P = .009$).

Distribution of Genetic Polymorphisms for Thymidylate Synthase

Another area of growing interest in this field is the observation that the activity of antifolate agents may be related to germline differences in the expression of the target enzyme, thymidylate synthase (TS). Pemetrexed, though a multitargeted antifolate, appears to have its primary activity at TS. TS expression is controlled in part by the *TS* enhancer region (*TSER*) within the 5′ untranslated region of the *TS* gene. Recent work has shown that the *TSER* is polymorphic with significant ethnic variation and relates to the activity of the agents. Tandem repeats of 28 base pairs have been identified,

Table 2 Geographic Differences in the Incidence of TSER*3 Polymorphism[35]

Population	Individuals Homozygous for *TSER*3* (%)
White	28
African-American	24
Southwest Asian	40
Chinese	67

and expression of the gene is increased with additional repeats. A triple tandem repeat (*TSER*3*) demonstrates 2.6-fold greater expression than the double repeat (*TSER*2*). There is considerable variation in this polymorphism both within and between ethnic groups (Table 2).[35]

Increased expression of this enzyme can alter both the activity and pharmacology of folate antagonist agents. For example, the activity of 5-fluorouracil activity in colon cancer is influenced by the *TSER* polymorphism.[36] Patients homozygous for *TSER*3* show increased intratumoral levels of TS protein. Higher levels of TS are associated with poorer response rates and survival. In lung cancer, there is evidence from Japanese studies that elevated TS levels correlate with increased proliferation and decreased sensitivity to antifolate agents (specifically 5-fluorouracil).[37,38] Preliminary data indicate that *TS* gene polymorphisms are prognostic for patients treated with platinum-based chemotherapy.[39] Studies are currently in preparation to determine whether *TS* gene polymorphisms are a predictive or prognostic factor (or both) for treatment with pemetrexed in NSCLC.

Conclusion

Second- and third-line treatments have now emerged as a standard of care throughout the world. Regulatory agencies in the United States and EU have approved docetaxel, pemetrexed, and erlotinib for second-line use. Japan was the first country to approve an EGFR TKI (gefitinib) for second-line use. There appears to be a substantially greater response to both gefitinib and erlotinib in Japan, but also a significant risk of life-threatening pneumonitis. Moreover, this variation in efficacy and side-effect profile appears to be present in other Asian populations. These ethnic differences may be surrogates for differences in genetic aspects of drug metabolism or potential differences in tumor susceptibility. The findings of a recent 'common arm' study performed in the United States and Japan in first-line therapy, as well as the studies of the two EGF TKIs, clearly demonstrate that the benefits and risks of anticancer agents may differ between populations. It is clear that the benefits and risks of anticancer agents differ between populations.

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ORIGINAL ARTICLE

Haplotype structures of the *UGT1A* gene complex in a Japanese population

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Genetic polymorphisms of UDP-glucuronosyltransferases (UGTs) are involved in individual and ethnic differences in drug metabolism. To reveal co-occurrence of the *UGT1A* polymorphisms, we first analyzed haplotype structures of the entire *UGT1A* gene complex using the polymorphisms from 196 Japanese subjects. Based on strong linkage disequilibrium between *UGT1A8* and *1A10*, among *1A9*, *1A7*, and *1A6*, and between *1A3* and *1A1*, the complex was divided into five blocks, *Block 8/10*, *Block 9/6*, *Block 4*, *Block 3/1*, and *Block C*, and the haplotypes for each block were subsequently determined/inferred. Second, using pyrosequencing or direct sequencing, additional 105 subjects were genotyped for 41 functionally tagged polymorphisms. The data from 301 subjects confirmed the robustness of block partitioning, but several linkages among the haplotypes with functional changes were found across the blocks. Thus, important haplotypes and their linkages were identified among the *UGT1A* gene blocks (and segments), which should be considered in pharmacogenetic studies.
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Keywords: UGT1A; single nucleotide polymorphism; linkage disequilibrium; haplotype

Introduction

Glucuronidation, catalyzed by UDP-glucuronosyltransferases (UGTs), is one of the critical steps in the detoxification and elimination of various endogenous and exogenous compounds.[1,2] As for the genes coding UGTs, two subfamilies, *UGT1* and *UGT2*, have been identified in humans. The human *UGT1A* gene complex spans approximately 200 kb, is located on chromosome 2q37, and consists of nine active and four inactive exon 1 segments (in the following segment order: *UGT1A12P*, *1A11P*, *1A8*, *1A10*, *1A13P*, *1A9*, *1A7*, *1A6*, *1A5*, *1A4*, *1A3*, *1A2P*, and *1A1*) and common exons 2–5 (Figure 1). One of the nine active exon-1's (namely, *1A1* and *1A3–1A10*) can be used in conjunction with the common exons.[2,3] The UGT1A N-terminal domains (encoded by the exon-1's) determine the substrate-binding specificity and the C-terminal domain (encoded by exons 2–5) is important for binding to UDP-glucuronic acid.[1] Thus, the exon 1 segments confer the substrate specificity of UGT1A isoforms,[4] and the 5′-flanking region (and possibly the 3′-flanking region) of each exon 1 is acknowledged to independently regulate the expression of each isoform.[3,4]

A number of genetic polymorphisms including single nucleotide polymorphisms (SNPs) in *UGT1As* have been identified and published on the UDP glucuronosyltransferase home page (http://som.flinders.edu.au/FUSA/ClinPharm/UGT/allele_table.html). Some of these polymorphisms are known to affect glucuronidation rates.[5–14] Regarding *1A1*, a TATA box variant (−40_−39 insTA: **28* allele), increases the risk of irinotecan-induced toxicity via decrease

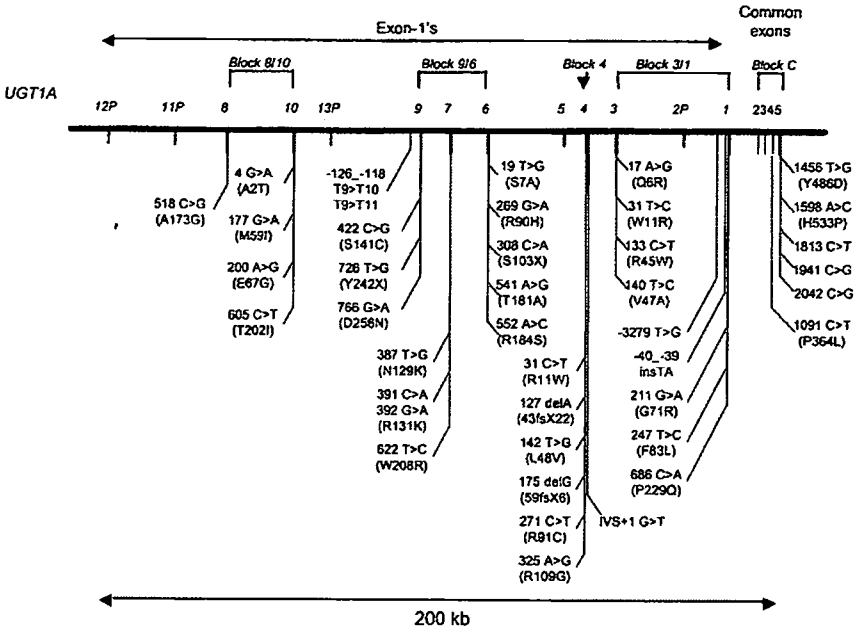

Figure 1 The organization of the human *UGT1A* gene complex and the polymorphisms used for genotyping. The human *UGT1A* gene complex (nine active and four inactive exon-1's, and common exons 2–5) spans approximately 200 kb. Four pseudogenes (*1A2p*, *1A11p*, *1A12p*, and *1A13p*), and *1A5*, which mRNA expression has not yet been detected in any tissue, were not analyzed in this study and are depicted by the small and gray bars. The complex was divided into five blocks, *Block 8/10*, *Block 9/6*, *Block 4*, *Block 3/1*, and *Block C*.

in detoxicating glucuronidation of 7-ethyl-10-hydroxy-camptothecin (SN-38), an active metabolite of irinotecan.[5] Another *1A1* polymorphism, 211G>A (G71R: **6* allele), also shows reduced activity to SN-38.[7,15] In addition, it has been reported that the *1A7* alleles, **2* (387T>G (N129K), 391C>A and 392G>A (R131K)), **3* (N129K, R131K, and 622T>C (W208R)) and **4* (W208R) show reduced activities towards benzo(a)pyrene metabolites: for SN-38 glucuronidation, **3* and **4*, but not **2*, are less active.[6,7]

Haplotypes, linked combinations of SNPs on a chromosome, have the advantage of providing more useful information on phenotype–genotype links than individual SNPs.[16] Co-occurrence of the SNPs or segmental haplotypes with functional changes in the *UGT1A* complex could lead to a cooperative alteration in glucuronidation activity. Kohle *et al.*[17] reported close linkages among *1A1*28*, *1A6*2* (T181A/R184S), and *1A7*3* (N129K/R131K/W208R) in Caucasians and Egyptians. Moreover, a recent analysis has shown that the low-activity alleles, *1A7*2* and **3*, were completely associated with the *1A9* −126_−118 T9 allele, whereas the high-activity *1A7*1* allele was linked with the −126_−118 T10 allele (*1A9*22*: high expression) in Americans.[18] However, there is no haplotype analysis with a high SNP density for the entire *UGT1A* complex, especially for Asian populations, which includes the Japanese.

Previously, we have reported the segmental haplotype structures for *1A1*, *1A4*, and *1A6* exon-1's in Japanese subjects.[19–21] In this study, additional first exons (*1A3*, *1A7*, *1A8*, *1A9*, and *1A10*) and their surrounding promoter or intronic regions were sequenced for the same 196 Japanese subjects as used for the analysis of *1A1*, *1A4*, and *1A6*, and the haplotypes for the *UGT1A* complex were inferred in linkage disequilibrium (LD) blocks, *1A8-1A10*, *1A9-1A7-1A6*, and *1A3-1A1*. Then, the tagged polymorphisms with functional changes were genotyped for additional 105 Japanese subjects. Finally, several linkages among the block haplotypes were inferred in a total of 301 subjects and compared with those of other ethnic groups.

Results

UGT1A8, 1A10, 1A9, 1A7, *and* 1A3 *polymorphisms detected in a Japanese population*

All the exon-1's and their flanking regions of *UGT1A8*, *1A10*, *1A9*, *1A7*, and *1A3* were sequenced in 196 Japanese subjects (108 arrhythmic patients and 88 cancer patients). As for *1A6*, *1A4*, *1A1*, and common exons 2–5, their SNPs and segmental haplotypes have already been reported.[19–21] *UGT1A5* was omitted from the current analysis because the expression of *1A5* mRNA has not been shown in any tissue.[2] AF297093.1 (GenBank accession number) was used as the *UGT1* reference sequence. All the allele frequencies were in Hardy–Weinberg equilibrium. No statistically significant differences in allelic frequencies of the detected SNPs were found between the subjects with the different disease types ($P \geq 0.05$ by χ^2 test or Fisher's exact test). Thus, the data for all subjects were analyzed as one group.

In *1A8*, seven SNPs, including two novel synonymous ones, were detected (Figure 2). The known nonsynonymous